Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biological Control 35 (2005) 104–114
Biological Control
www.elsevier.com/locate/ybcon

Strain selection of a fungal entomopathogen, *Beauveria bassiana*, for control of plant bugs (*Lygus* spp.) (Heteroptera: Miridae) ☆

Jarrod E. Leland [a,*,1], Michael R. McGuire [b,1], Julie A. Grace [c], Stefan T. Jaronski [c], Mauricio Ulloa [b], Young-Hoon Park [d], Ronald D. Plattner [e]

[a] *USDA, Agricultural Research Service, Southern Insect Management Research Unit, Stoneville, MS 38776, USA*
[b] *USDA, Agricultural Research Service, Shafter Research and Extension Center, Shafter, CA 93263, USA*
[c] *USDA, Agricultural Research Service, Northern Plains Agricultural Research Center, Sidney, MT 59270, USA*
[d] *Department of Crop Genetics, University of California, Davis, CA, USA*
[e] *USDA, Agricultural Research Service, Mycotoxin Research Unit, Peoria, IL 61820, USA*

Received 21 December 2004; accepted 7 June 2005
Available online 24 August 2005

Abstract

Beauveria bassiana (Balsamo) isolates from *Lygus hesperus* (Knight) and *Lygus lineolaris* (Palisot de Beauvois) are being evaluated as potential microbial control candidates for both *Lygus* species. Based on previous work with a large collection of isolates from both *Lygus* species, eight isolates were evaluated for characteristics relevant to mycoinsecticide development. These characteristics included pathogenicity to the two *Lygus* spp., in vitro conidia production, temperature growth optima, tolerance to solar radiation, and production of beauvericin. Comparisons were made with a commercial isolate (GHA). Isolates from *L. hesperus* and *L. lineolaris* were more pathogenic than GHA with many being more than an order of magnitude more pathogenic based on LC_{50} values. All isolates from *L. hesperus* and two from *L. lineolaris* grew faster at 32 °C than GHA. The isolates selected from *L. hesperus* were the most tolerant to exposure to simulated solar radiation. Isolates from *L. lineolaris* and *L. hesperus* were most closely related to other isolates from their same host species and all of these isolates were only distantly related to GHA. Interestingly, one isolate from *L. lineolaris* in Mississippi was more closely related to an isolate from *L. lineolaris* in Arkansas than to other isolates from *L. lineolaris* from the same county in Mississippi. Optimizing conidia production conditions for a select isolate may be necessary for practical development of a select isolate from *Lygus* spp. as a commercial mycoinsecticide.

Keywords: *Beauveria bassiana*; *Lygus lineolaris*; *Lygus hesperus*; Tarnished plant bug; Western tarnished plant bug; Microbial control

1. Introduction

Lygus plant bugs (Heteroptera: Miridae) are significant pests of a wide range of fruit, vegetable, seed, and fiber crops. Annual economic losses including yield loss and control costs from *Lygus* spp. in US cotton alone have reached nearly $100 million in recent years (Williams, 2004). Control options are primarily limited to a few insecticide classes and resistance is of increasing concern (Grafton-Cardwell et al., 1997; Snodgrass, 1996; Snodgrass and Scott, 2002). Entomopathogenic fungi may provide options, particularly if strategically used to control populations developing on alternate hosts (Goodell et al., 2000; Snodgrass et al., 2000).

Two commercial *Beauveria bassiana*-based mycoinsecticides have been evaluated against *Lygus lineolaris*

☆ The mention of firm names or trade products does not imply that they are endorsed or recommended by the US Department of Agriculture over other firms or similar products not mentioned.

* Corresponding author. Fax: +1 662 686 5421.
E-mail address: jleland@ars.usda.gov (J.E. Leland).

[1] Contributed equally to this paper.

1049-9644/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.biocontrol.2005.06.005

(Palisot de Beavois) and *Lygus hesperus* (Knight) with mixed success. Mycotrol (Emerald Bioagriculture, Lansing, MI) had moderate activity against *L. lineolaris* in cotton, particularly when applied with low rates of imidacloprid (Steinkraus and Tugwell, 1997). However, control of *L. hesperus* in alfalfa with Mycotrol was relatively poor, which may in part be attributed to poor coverage, particularly at bloom (Noma and Strickler, 1999). Naturalis-L (Fermone Corp.) provided relatively poor control against *L. lineolaris* in cotton (Snodgrass and Elzen, 1994).

Selecting more pathogenic isolates may improve the efficacy of mycoinsecticides for *Lygus* control. In a study comparing the pathogenicity of 32 fungal isolates from six genera to *L. lineolaris*, 21 isolates caused statistically similar *L. lineolaris* mortality as the GHA isolate. Mortality was higher, although not statistically significant, for 15 of these isolates (Liu et al., 2002). Four *B. bassiana* isolates and a *Metarhizium anisopliae* isolate were selected for further studies based on pathogenicity in single dose screenings and conidia production for multiple dose assays. Three of these isolates (*B. bassiana* ARSEF 1394, 5665, and *M. anisopliae* 3540) were significantly more pathogenic than GHA. It is worth noting that the two *B. bassiana* isolates were non-indigenous isolates, which increases the difficulty in commercially developing them in the US Bioassays were also conducted at 20 °C, which may select for isolates with temperature growth optima outside the desired range for control conditions of *Lygus* spp. in warm regions.

By obtaining isolates from *Lygus* spp. in warmer regions where *Lygus*spp. are major pests of cotton, strawberries, and other crops, it may be possible to select isolates better suited to infecting *Lygus* spp. in these areas. *B. bassiana* (ARSEF 3769), which was isolated from *L. lineolaris* in Arkansas, caused high *L. lineolaris* mortality in caged field trials (Steinkraus and Tugwell, 1997). A survey of natural *B. bassiana* infection levels in *L. hesperus* populations of the San Joaquin Valley (SJV), California over a three-year period demonstrated relatively high natural infection levels ranging from 0 to 50% with a trend toward increasing infection levels throughout the season (May through November). Surprisingly, some populations demonstrated relatively high infection levels (>20%) in July, when temperatures exceed 40 °C (McGuire, 2002). A similar survey was conducted to determine natural infection levels in *L. lineolaris* populations of the Mississippi delta and hill regions (Leland and Snodgrass, in press). Although natural infection levels in *L. lineolaris* from Mississippi were approximately 30 times lower than those observed in *L. hesperus* from California (McGuire, 2002), 20 *B. bassiana* isolates were obtained in 2002–2003 (Leland and Snodgrass, in press). Isolates from *L. hesperus* were screened for pathogenicity to *L. hesperus*, in vitro conidia production, radial growth rates of colonies at 28–37 °C, and genetic relatedness among these 80 isolates was determined to select a few candidate isolates for further development (McGuire et al., 2005). The 20 isolates from *L. lineolaris* were screened for pathogenicity to *L. lineolaris*, in vitro conidia production, conidia germination at 35 °C, and survival under simulated solar radiation (Leland, unpublished data). From these studies, three isolates from *L. hesperus* and four isolates from *L. lineolaris* were selected for further examination. In addition, GHA and ARSEF 3769 were included in the tests.

The objectives of this study were to compare characteristics relevant to commercial development, and genetic relatedness among the selected isolates. The characteristics determined for each isolate include: (1) in vitro conidia production on barley agar and in diphasic fermentation, (2) pathogenicity to both *L. lineolaris* and *L. hesperus*, (3) radial growth rates at 28–37 °C, (4) conidia survival under simulated solar radiation, (5) beauvericin production in vitro and in vivo, and (6) genetic relatedness.

2. Materials and methods

2.1. Fungal culture history

Nine *B. bassiana* isolates were evaluated in this study (Table 1), which were cultured in three different laboratories for various aspects of this research as described below. Culture conditions for experiments conducted in California, which included bioassays against *L. hesperus*, radial growth rates at 28–37 °C, genetic relatedness, and beauvericin production were described previously (McGuire et al., 2005). Briefly, isolates were grown on SDAY, harvested and enumerated with a hemocytometer. Conidia viability was assessed following incubation for 16 h in potato dextrose broth (Sigma) just prior to use in experiments. Assays were conducted before and after single conidia isolations were made from each of the nine isolates. However, genetic examination (see below) determined that there was no difference between the cultures before and after single sporing, which suggested that the original cultures (each from a single insect) were clonal.

Culture conditions for experiments conducted in Mississippi, which included bioassays against *L. lineolaris*, in vitro conidia production on barley agar, and solar simulation experiments were conducted as follows. Approximately 6000 *L. lineolaris* adults and nymphs were collected from native populations at 52 locations and 20 counties from wild host plants in May through November 2002 and 2003 (Leland and Snodgrass, in press). Insects were incubated individually on broccoli until death or for 14 days. Cadavers were surface sterilized in 10% ethanol and 0.525% sodium hypochlorite (10% household bleach) and incubated in 96-well plates

Table 1
Beauveria bassiana isolates collected from San Joaquin Valley, California, populations of *L. hesperus* and from the Delta region, Mississippi populations of *L. lineolaris*

Designation	Alternate designation	Host spp.	Host plant	Year collected	Julian day	County (State)
WTPB1	44-03	*L. hesperus*	Alfalfa	2001	219	Kern (CA)
WTPB2	17-41	*L. hesperus*	Alfalfa	2001	179	Tulare (CA)
WTPB3	54-43	*L. hesperus*	Alfalfa	2001	219	Kern (CA)
TPB1	NI1	*L. lineolaris*	Erigeron	2002	101	Washington (MS)
TPB2	NI6	*L. lineolaris*	Vervain	2002	153	Washington (MS)
TPB3	NI8	*L. lineolaris*	Horseweed	2002	210	Washington (MS)
TPB4	NI9	*L. lineolaris*	Pigweed	2002	269	Washington (MS)
3769	ARSEF 3769	*L. lineolaris*	Alfalfa	1992	168	Little River (AR)
GHA[a]	ARSEF 6444 ATCC 74250	Laboratory reisolation from *Locusta migratoria* March 2000; Mycotech Corporation BB726				

[a] GHA is the designation for the isolate within the commercial product Mycotrol (Emerald BioAgriculture).

(0.4 mL wells, Steriline) containing 170 μL of water agar (15 g L^{-1} agar) in each well for 72 h for sporulation (Noma and Strickler, 2000). Sporulating insects were suspended in 1.5 mL of 0.01% Tween 80 and vortexed for 30 s. The conidia suspensions were spread on agar selective for *B. bassiana* and *Metarhizium* spp. (Doberski and Tribe, 1980). The 21 isolates from *L. lineolaris* that grew on this selective media were harvested after 2 weeks in 0.01% Tween 80 and 15% glycerol, diluted to 5×10^6 conidia mL^{-1}, and stored in 1 mL aliquots at −80 °C to be used as inocula for future experiments; four of these isolates were selected for the present study based on initial screening (Leland, unpublished data). GHA (ARSEF 6444) and ARSEF 3769 were obtained from the ARSEF collection and starter cultures were grown on Sabouraud dextrose agar (Sigma Chemical, St. Louis, MO) with 2 g L^{-1} yeast extract (Sigma) (SDAY) for 2 weeks at 25 °C. Conidia suspensions (5×10^6 conidia mL^{-1}) in 0.01% Tween 80 (polyoxyethylenesorbitan monooleate, Sigma) and 15% glycerol (Sigma) were made from these cultures and frozen in 1.5 mL aliquots at −80 °C to serve as inocula for future experiments.

From experiments examining pathogenicity, environmental stress tolerance, and conidia production (McGuire et al., 2005; Leland, unpublished data), three isolates from *L. hesperus* and four from *L. lineolaris* were selected for further study. Specific details on isolate numbering and collection information are presented in Table 1.

2.2. Mass production

Isolates were initially screened for spore production potential on barley agar and later, select isolates were evaluated in a biphasic system that simulates industrial-scale production. Isolates were screened for spore production potential on barley agar in 250 mL flasks. Aliquots of conidia (100 μL containing 5×10^5 conidia) were used to inoculate four replicate 250 mL Erlenmyer flasks containing 50 mL of barley agar (30 g L^{-1} pulverized hulled organic barley [Organic Kingdom, Orem, UT), 1 g L^{-1} yeast extract (Sigma), and 15 g L^{-1} agar (Sigma)]. Flasks were capped with 45 mm foam plugs (Scimart, St. Louis, MO) and incubated at 25 °C for 10 days. Conidia were harvested by adding 20 mL of 0.01% Tween 80 plus 6 mm glass beads and shaking at 400 rpm for 5 min (New Brunswick Gyratory G-76 Orbital Shaker, Edison, NJ). Resulting conidia suspensions were filtered through two layers of cheese cloth to remove mycelia and beads and then enumerated by hemacytometer (Brightline Improved Neubauer Ruled; Hausser Scientific, Horhsam, PA). These methods were repeated twice and also used to produce conidia suspensions for bioassays with *L. lineolaris* and solar simulation experiments.

Spore production potential was determined for TPB3, TPB4, WTPB2, and WTPB3 in a biphasic culture system that simulates industrial-scale production systems (Bradley et al., 1992, 2002). Flasks (1 L) of liquid CSYE medium (40 g L^{-1} glucose, 10 g L^{-1} KNO_3, 5 g L^{-1} KH_2PO_4, 1 g L^{-1} $MgSO_4$, 0.05 g L^{-1} $CaCl_2$, and 2 g L^{-1} yeast extract), were inoculated with conidia from SDAY media and incubated for 3–4 days at 25–26 °C and 150 rpm. These liquid cultures were then used to inoculate autoclaved, pearled barley (Minnesota Grain, Eagan, MN) in sterilized, plastic, vented mushroom spawn bags (Unicorn Implement and Manufacturing, Commerce, TX). The barley was first autoclaved at 103 kPa for 20 min kg^{-1}, and then cooled before transfer to a spawn bag. Three- to four-day-old liquid culture of each *Beauveria* isolate was mixed by hand with the substrate, under aseptic conditions, at a ratio of 1:2 (v/w) and the bag heat-sealed. Three replicate batches of 300 g autoclaved barley each were prepared for each isolate. Solid substrate fermentation was conducted for 8 days at 26–27 °C in constant darkness. Cultures were observed daily and crumbled by hand within spawn bags as

needed to prevent binding of the substrate and provide aeration throughout the culture substrate. Whole cultures were then transferred to plastic chambers where they were dried for 7 days at 28 °C at an air exchange rate of 0.08 vvm for the first three days then 0.04 vvm for another 5 days. Conidia from each replicate batch were individually harvested by mechanical sieving through 20 and 100 mesh sieves in an ultrasonic sieve shaker (AS200, Retsch, Newton, PA) under identical conditions. Conidial fractions greater than 20 mesh (0.85 mm), between 20- and 100-mesh (0.15–0.85 mm), and >100 mesh (0.15 mm) were evaluated separately. Conidial numbers in each fraction were determined by suspending a 0.1 g sample in 0.05% Silwet L77, diluting serially as appropriate, and counting in a hemocytometer using 400× phase-contrast microscopy. Three replicate samples and counts were made for each fermentation batch. Conidial viability of each fraction after harvest was based on percent conidial germination on half-strength SDAY after 16–18 h at 28 °C. Only conidia with hyphal pegs greater than conidial diameter were considered germinated. At least 400 conidia were examined for each germination test. Spores from the 100-mesh fraction were dried at 15% RH and 25 °C to less than 10% moisture for storage.

2.3. Bioassays with L. hesperus

Lygus hesperus were reared in the laboratory as described by McGuire et al. (2005). Adults that had emerged over a two-day period were used in all experiments.

Conidia suspensions were applied to *L. hesperus* in 5 mL of 0.01% Silwet in a spray tower equipped with a TG 0.4 solid cone nozzle (Spraying Systems) as described by McGuire et al. (2005). Each bioassay was done with four concentrations of conidia to produce 8.1×10^1, 8.1×10^2, 8.1×10^3, and 8.1×10^4 conidia cm^{-2}. Twenty insects were anesthetized with CO_2 and transferred to a 9 cm diameter Petri dish lined with dry filter paper. The dish was then transferred to the tower, insects were sprayed and then transferred to small plastic vials (5 dram) containing a piece of green bean, and each vial capped with a foam plug. Beans were changed every 2–3 days and insects were held for 7 days at 28 °C, 16L:8D or until they died. Insects that died at 1day post treatment were excluded from analysis. As insects died, they were transferred to 1.5% water agar and held for at least three days for sporulation to occur. Controls consisted of *L. hesperus* treated with CO_2 and sprayed with 0.01% Silwet L77. All nine isolates were assayed together and the experiment was repeated three times.

Estimates of LC_{50} values for mortality and sporulation (SC_{50}) at 7 days post treatment were made using probit analysis (Proc Probit, SAS) (Finney, 1971). Estimates of SC_{50} values represented the median concentration which resulted in sporulation of 50% of the insects. Variances and covariances were adjusted for over dispersion based on a Pearson's χ^2 test but χ^2 values for slope are presented rather than for goodness-of-fit, since heterogeneity χ^2 values are often misinterpreted as representing that trends significantly fit the data. Estimates of LC_{50} and SC_{50} values were for individual isolates among the three bioassays were not significantly different so data were combined for determination of LC_{50} and SC_{50} values. Estimates of LC_{50} and SC_{50} values were compared on the basis of overlapping 95% confidence intervals. Control mortality was less than 20% in all three assays. Estimates of LT_{50} values at each conidia concentration were made using survivorship analysis (Proc Lifereg, SAS) using right censored data for insects surviving beyond the 7 days incubation period. Standard errors were generated independently for each isolate at each spore concentration by Proc Lifereg, SAS, and therefore LT_{50} values were compared based on pairwise *t* tests.

2.4. Bioassays with L. lineolaris

Insects used in initial bioassays (prior to single conidia isolation) were adults of unknown age collected from a mixed stand of horseweed [*Conyza canadensis* (L.) Cronquist] and vervain (*Verbena brasiliensis* Vellozo) on July 11, 2003 and held 48 h at 25 °C, 80 ± 5% relative humidity, with a 14:10 L/D photoperiod prior to the bioassay. Insects used in the bioassay of isolates following single conidia isolation were collected from pigweed (*Amaranthus* spp.) on October 3, 2003 as adults of unknown age and held 48 h prior to the bioassay under the same conditions. Bioassays were conducted for each isolate once prior to single spore isolation and once following single spore isolation using three replicates in each bioassay. All isolates were included in both bioassays. Three replicates of 10 insects per conidia concentration were held in 9 cm Petri dishes moistened with 0.7 mL of water. Conidia suspensions of isolates were applied to *L. lineolaris* in 1 mL of 0.01% Tween 80 using a Potter spray tower to produce 1.7×10^2, 1.7×10^3, 1.7×10^4, and 1.7×10^5 conidia cm^{-2}. Sixteen replicates of 10 *L. lineolaris* were exposed to 0.01% Tween 80 in the spray tower to serve as controls in each bioassay. After being sprayed, insects were transferred to individual 30 mL medicine cups each containing a single broccoli floret and capped with a 45 mm foam plug (Scimart, St. Louis, MO). Insects were incubated at 25 °C, 80 ± 5% relative humidity, and a 14:10 L/D photoperiod. Mortality was determined daily and broccoli was changed at 2 days intervals. After 10 days incubation, all of the insects in the bioassay were surface sterilized in 10% ethanol and 0.525% sodium hypochlorite and incubated in 96-well plates (0.4 mL wells, Steriline) containing 170 μL of water agar (15 g L^{-1} agar) in each well for 72 h for sporu-

lation (Noma and Strickler, 2000). Estimates of LC_{50} and SC_{50} values at 7 days post treatment were made using probit analysis (Proc Probit, SAS) for each bioassay and data were combined as above. Overall control mortality at 7 days post treatment was 30%. Estimates of LT_{50} values at each conidia concentration were made using survivorship analysis (Proc Lifereg, SAS) as stated above.

2.5. Temperature tolerance

Isolates tested in this study were evaluated for their growth rates at different temperatures. Conidia from each isolate were spread onto SDAY in 9 cm diameter Petri plates and allowed to grow for 3 days at 28 °C. At that time a 9 mm diameter brass cork borer was used to extract a plug from the edge of the actively growing isolate. The plug was transferred to the center of a 9 cm diameter Petri plate containing 15 mL SDAY. All plates were wrapped with two layers of stretched parafilm. Three plates were established for each isolate for each temperature. Plates were placed in constant temperature, dark growth chambers programmed at 28, 32, 35, and 37 °C, each monitored for temperature by a HOBO temperature recorder programmed for 30 min readings. Every day, plates were quickly (<1 min) examined for cracks in the parafilm and the parafilm was replaced as necessary (especially at the two higher temperatures). At approximately 5 days intervals, the diameter on two radial axes was measured with a caliper and the average diameter was recorded. After 28 days the experiment was terminated and total growth per day was calculated. Because no isolates grew at 35 or 37 °C, the experiment was repeated only at 28 and 32 °C using incubators previously used for different temperatures in the first experiment. Results were analyzed by ANOVA and means were separated by protected least significant difference (Statistix).

2.6. Exposure to artificial sunlight

Artificial sunlight experiments evaluated the survival of *B. bassiana* conidia. Artificial sunlight was provided by a solar simulator (Model 91193, Oriel Corp.) equipped with a 1000 W Xenon Arc Lamp corrected with air mass 0 and air mass 1 filters to simulate direct noon sunlight. Three replicate suspensions (10 mL of 1×10^7 conidia mL^{-1}) of each isolate from individual barley agar cultures were deposited onto nylon membrane filters by vacuum filtration to remove water (Magna, 0.45 μm pore size, 47 mm diameter) and exposed to 1, 2, 4, or 8 h of simulated solar radiation at 19.1 ± 0.5 mW cm^{-2} from 200 to 3000 nm (IL 1400A Radiometer/Photometer with an SEL623 Thermopile Detector, International Light, Newburyport, MA). A separate nylon membrane filter containing deposited conidia was used for each exposure time period for each replicate. The exposure times corresponded to 6.9, 13.8, 27.5, and 55.0 J cm^{-2}, respectively. Filters were held at 4 °C prior to exposure and appeared dry at the time of exposure to simulated solar radiation. After exposure, conidia were rehydrated for 1 h at 100% RH and suspended in 2 mL of 0.04% Tween 80. Samples (100 μL) of the conidia suspensions were spread onto surfaces of two 60 mm Petri dishes containing germination agar [20 g L^{-1} malt extract (Sigma); 15 g L^{-1} agar (Sigma); 0.02 g L^{-1} Benlate 50WP; 0.2 g L^{-1} chloramphenicol (Sigma); 200,000 U penicillin L^{-1} (Sigma); 0.2 g L^{-1} streptomycin (Sigma)]. Benomyl was added to the media to prohibit growth of fast germinating conidia from masking slow germinating conidia (Milner et al., 1991). Conidia were killed with lactophenol acid fuschin mounting media (phenol 20 g; lactic acid 20 g; glycerol 40 g; acid fuschin 0.1 g; water 20 mL) after 24 and 48 h incubation periods. Glass cover slips were pressed onto the surface of the germination agar and percentage conidia germination was determined for 200 conidia from each replicate at 1000× magnification (Goettel and Inglis, 1997). The entire experiment was repeated once. Estimates of LT_{50} values for loss in spore germination over exposure time (ln) to artificial sunlight were made using probit analysis (Proc Probit, SAS).

2.7. Beauvericin production

The following three sources of *B. bassiana* were analyzed for beauvericin concentration: (1) *B. bassiana* isolates were grown in PD-broth for 9 days at 28 °C, frozen and sent via overnight service from Shafter, California to Peoria, Illinois; (2) conidia scraped from SDAY as previously described; (3) cadavers of infected *L. hesperus* were produced by treating adults with 1.8×10^4 conidia cm^{-2} of each isolate, incubating for 7 days and then holding for sporulation as described in bioassays with *L. hesperus*, above; (4) GHA technical grade powder (TGAI) Lot # 010701.

Cultures from PD-broth were stored frozen prior to analysis. These samples were filtered through a 0.2 μm filter to remove conidia and other debris and 10 μL of this filtrate was injected directly into a quadrupole ion trap mass spectrometer coupled to a liquid chromatographer (LC/MS) (Thermo Finnigan LCQ Deca). The LC column was a 150 cm × 0.3 mm 5 μm Intersil ODS3 column (Metachem). The conditions were isocratic at 0.3 ml/min of methanol/water (83/17). The column output was coupled directly to the MS via the API interface in the electrospray (esi) mode. The spray voltage was 4.5 kV. In the full scan MS mode, the instrument was scanned from 250 to 1150. The base peak in the spectrum of beauvericin was *m/z* 806 (M + Na)+. Other signals observed were *m/z* 784 (MH+) and 801 (M + 18-probably NH_4). For quantification, *m/z* 806 was selected as the parent ion with an ionization with

of 2 amu. A normalized collision energy of 35% was used at an activation Q of 0.25 and an activation time of 30 ms. The scan was from m/z 220 to 850 and major daughters were observed at m/z 645, m/z 545, and m/z 384. Quantification was based on the abundance of the most abundant daughter (m/z 645) and confirmation was based on the relative abundances of the three daughters. Pure conidia and sporulating *L. heseprus* cadavers were extracted with 1:1 acetonitrile/water at 0.2 mL of solvent per mg of sporulating insect weight. Extracts were then diluted 10,000-fold with 1:1 acetonitrile/water and 10 mL of the diluted extract was injected directly into the LC/MS and analyzed as described above. GHA technical grade samples (5 mg) were suspended in 1 mL of 1:1 acetonitrile/water and a 10 mL aliquot injected directly into the LC/MS and analyzed as described above.

2.8. Genetic relatedness

To estimate genetic relatedness of the isolates approximately 50 mg of mycelia were collected from each isolate, and total genomic DNA was extracted with Dneasy Plant Mini Kit (QIAGEN, Valencia, CA) following the manufacture's instructions. Seven PCR primer pairs previously developed (Rehner and Buckley, 2003), which flank simple sequence repeats (SSR), were used to screen the *B. bassiana* isolates. PCR primers were synthesized by Proligo (Boulder, CO). PCR amplification was performed in a total volume of 20 μl containing 20 ng of template DNA, 0.1 μM of each primer (forward and reverse), 1× PCR buffer, 0.2 mM of dNTPs, and 1 U Taq polymerase (Amplitaq, Applied Biosystem, Foster city, CA) with cycling profile of 1 cycle of 2 min at 94 °C, 10 cycles of 15 s at 94 °C, 30 s at 60 °C (step −0.5 °C/cycle for cycles 2–10), and 1 min at 72 °C, 35 cycles of 15 s at 94 °C, 30 s at 55 °C, and 1 min at 72 °C. PCR products were first checked and separated on a 3% Super Fine Resolution (SFR) agarose (Amresco, Solon, OH) gel containing 1× TBE at 80 V for 4–5 h, and visualized by Alpha imager (Alpha Innotech Corporation, San Leandro, CA) software v. 5.5 after staining with ethidium bromide. After primer pairs were amplified and resulted in discrete PCR banding patterns, primers were again synthesized and labeled with RED Well Dye D2, D3, D4 for their use with a CEQ 8000 DNA Analysis System (Beckman Coulter Company). The seven primer pairs amplified DNA fragments from a SSR locus in all isolates examined. Allele-peak names were designated by locus name (primer ID) followed by the number 1, 2, 3, 4, 5, 6, or 7, where 1 is the highest molecular weight allele, 2 is the second highest molecular weight, etc.

To estimate genetic similarity among the isolates, pairwise genetic similarity coefficients were calculated based on Jaccard's similarity coefficient (Jaccard, 1908) to evaluate the pattern of genetic variation among strains. The similarity coefficient was calculated as $[a/(n-d)]$ for each pair of strains, where a is the number of alleles for which a band-peak is present and d is the number of alleles for which the band-peak is absent, and n is the total number of alleles. A dendrogram was constructed using the unweighted pair group method average (UPGMA) clustering analysis of the genetic similarity coefficient matrices. All computations for the statistical analysis of the data and the projection of dendrograms were performed using the numerical taxonomy and multivariate analysis system (NTSYS-pc) version 2.1 (Rohlf, 2002).

3. Results and discussion

3.1. Conidia production

Isolates from *L. lineolaris* collected in Mississippi (TPB1, TPB2, TPB3, and TPB4) produced the greatest numbers of conidia on barley agar and conidia production of all other isolates on barley agar was similar (Table 2). However, the conidia production potential on barley agar did not translate into similar conidia production in scaled-up biphasic culture (Table 2). In biphasic culture, production was highest for GHA in all size fractions of conidia product and total conidia production. Among the isolates from *Lygus* spp., total conidia production was highest for TPB3, but the fraction that was recovered following passage through a 100 mesh sieve (<0.14 mm) was not significantly greater than any other isolate from *Lygus* spp. The sieve harvesting system was not equally efficient at harvesting conidia at various fractions from all isolates. For example, the percentages of total conidia produced that were collected following passage through a 100 mesh sieve (<0.14 mm) for GHA, WTPB1, WTPB3, TPB3, and TPB4 were 37 (±17), 25 (±26), 14 (±8), 54 (±6), and 21 (±9)%, respectively. The low conidial recovery of some of these isolates (e.g., WTPB1 and WTPB2) in the <100 mesh fraction was due to the nature of extensive mycelial growth on the solid substrate. The mycelia were bound tightly to the substrate, which made mechanical release of conidia difficult. Most of the conidia remained tightly bound to and within the whole culture and required dispersion in a highly efficient wetting agent, 0.05% Silwet L77. Solid substrate fermentation of strain GHA, on the other hand, created a very friable material from which conidia are readily released even in a relatively inefficient system such as mechanical sieving. Harvesting conidia from larger quantities of substrate might be improved with other harvesting methods (e.g., MycoHarvester, CABI, Biosciences). For example, harvesting efficiencies of GHA on a commercial scale at Emerald BioAgriculture routinely achieved efficiencies of >80% (Jaronski, unpublished data). Regardless, further work is needed to

Table 2
Conidia production (average ± SE) in vitro on barley agar and biphasic bag culture with liquid media (CSYE medium) followed by solid substrate (Barley)

Isolate	Flask culture Conidia ($\times 10^8$) cm^{-2}[A]	Conidia ($\times 10^{12}$) per kg substrate from biphasic culture			
		>0.85 mm fraction	0.15–0.85 mm fraction	<0.14 mm fraction[B]	Total
WTPB1	0.8 ± 0.2 c[C]	0.16 ± 0.09 b	0.023 ± 0.001 b	0.052 ± 0.05 b	0.23 ± 0.04 c
WTPB2	1.0 ± 0.2 c	0.24 ± 0.02 b	0.012 ± 0.011 b	0.028 ± 0.010 b	0.28 ± 0.16 c
WTPB3	0.3 ± 0.05 c	N/A	N/A	N/A	N/A
TPB1	2.8 ± 0.1 b	N/A	N/A	N/A	N/A
TPB2	2.7 ± 0.4 b	N/A	N/A	N/A	N/A
TPB3	10.2 ± 5.0 a	0.84 ± 0.10 b	0.093 ± 0.073 ab	1.1 ± 0.12 b	2.0 ± 0.23 b
TPB4	3.5 ± 0.5 ab	0.23 ± 0.08 b	0.048 ± 0.022 b	0.073 ± 0.033 b	0.36 ± 0.084 c
3769	1.3 ± 0.3 c	N/A	N/A	N/A	N/A
GHA	0.9 ± 0.2 c	4.69 ± 0.16 a	0.17 ± 0.04 a	2.80 ± 0.10 a	7.68 ± 0.88 a
F (*df*)	25 (8, 57)	20 (4, 13)	8 (4, 13)	18 (4, 13)	153 (4, 13)

[A] Total conidia production per cm^2 of surface area on 50 mL of barley agar in 250 mL flasks.
[B] The fraction of harvested conidia from biphasic culture that was smaller than 0.14 mm is similar to what would be harvested from a mechanical classification harvest system under industrial production conditions.
[C] Values within the same column followed by different letters are significantly different at the $\alpha = 0.05$ level (Proc GLM, Tukey's HSD; SAS).

determine if the biphasic system may be optimized in an economically practical manner to improve conidial production for isolates such as WTPB1 and WTPB2.

3.2. Pathogenicity to L. hesperus and L. lineolaris

Isolates from *L. lineolaris* and *L. hesperus* were generally more pathogenic than GHA based on LC_{50} and SC_{50} values (Table 3). All isolates from *L. lineolaris* except TPB1 were significantly more pathogenic to *L. lineolaris* than GHA based on LC_{50} and SC_{50} values (Table 3). Estimates of LC_{50} values for the most pathogenic isolate (TPB4) were approximately 20 times lower than that of GHA. Estimates of LC_{50} values for *L. lineolaris* treated with isolates from *L. hesperus* were not significantly different from GHA (Table 3). WTPB3 was more pathogenic based on mortality but sporulation of cadavers was very low in *L. lineolaris* (Table 3).

Estimates of LC_{50} values of *L. hesperus* exposed to all isolates from *Lygus* spp. were generally lower than that

Table 3
Mortality (LC_{50}) and sporulation (SC_{50}) values for adult *L. lineolaris* and *L. hesperus* seven days after exposure to *B. bassiana* strain GHA, ARSEF 3769, and isolates from *L. lineolaris* (TPB) and *L. hesperus* (WTPB)

Isolate	Slope[a] ± SE	LC_{50}[b]	95% CL	χ^2	$P > \chi^2$	Slope ± SE[c]	SC_{50}	95% CL	χ^2	$P > \chi^2$[c]
	Mortality of *L. lineolaris*					Sporulation on *L. lineolaris*				
GHA	0.60 ± 0.09	5.2	2.5–17.0	36	<0.001	0.87 ± 0.16	10.7	4.8–47.7	27	<0.001
3769	0.78 ± 0.14	0.49	0.22–1.0	33	<0.001	0.92 ± 0.09	1.5	0.75–3.5	36	<0.001
TPB1	0.64 ± 0.16	2.1	0.77–11.0	18	<0.001	0.69 ± 0.11	4.1	2.2–10.5	42	<0.001
TPB2	0.53 ± 0.14	0.43	0.094–1.7	13	<0.001	0.71 ± 0.09	1.9	1.1–4.0	47	<0.001
TPB3	1.0 ± 0.21	0.36	0.15–0.78	26	<0.001	0.81 ± 0.09	0.88	0.55–1.5	61	<0.001
TPB4	1.1 ± 0.16	0.27	0.14–0.50	45	<0.001	0.67 ± 0.16	1.2	0.49–3.3	28	<0.001
WTPB1	0.58 ± 0.23	1.7	0.28–264	7	0.009	0.37 ± 0.14	42.5	4.8–5E12	6.6	0.01
WTPB2	0.62 ± 0.18	1.6	0.41–22.7	10	0.001	0.64 ± 0.12	5.3	2.3–231	27	<0.001
WTPB3	0.64 ± 0.14	1.4	0.53–5.6	20	<0.001	0.30 ± 0.16	3.2E7	N/A[d]	3.3	0.07
	Mortality of *L. hesperus*					Sporulation on *L. hesperus*				
GHA	0.85 ± 0.13	0.47	0.21–1.2	40.2	<0.001	0.42 ± 0.06	0.61	0.38–1.0	42.3	<0.001
3769	1.2 ± 0.12	0.11	0.07–0.17	91.0	<0.001	0.36 ± 0.05	0.14	0.09–0.21	60.8	<0.001
TPB1	1.6 ± 0.27	0.14	0.07–0.27	32.8	<0.001	0.40 ± 0.07	0.13	0.07–0.25	30.4	<0.001
TPB2	0.90 ± 0.24	0.44	0.08–2.6	14.3	0.01	0.28 ± 0.06	0.52	0.23–1.2	19.6	<0.001
TPB3	1.2 ± 0.13	0.14	0.10–0.22	91.4	<0.001	0.33 ± 0.07	0.16	0.08–0.32	26.5	<0.001
TPB4	1.1 ± 0.22	0.11	0.04–0.28	24.9	<0.001	0.33 ± 0.05	0.17	0.07–0.37	51.4	<0.001
WTPB1	1.0 ± 0.20	0.58	0.22–1.7	27.1	<0.001	0.46 ± 0.05	0.79	0.36–1.8	76.7	<0.001
WTPB2	1.34 ± 0.14	0.26	0.18–0.38	86.9	<0.001	0.30 ± 0.09	0.37	0.25–0.55	12.5	<0.001
WTPB3	0.87 ± 0.24	0.52	0.11–3.9	13.3	<0.001	0.22 ± 0.10	0.56	0.19–2.0	4.5	0.03

[a] Slope for mortality or sporulation represents regression of proportion of mortality or sporulation versus log of conidia cm^{-2}.
[b] LC_{50} and SC_{50} values and 95% confidence limits (CL) expressed as conidia ($\times 10^4$) cm^{-2}, Proc Probit (SAS).
[c,d] χ^2 and $P > \chi^2$ values represent the probability of slope $\neq 0$, rather than fit to Probit. 95% CL not presented for $P > \chi^2$ values >0.02.

of GHA, but differences were only significant for ARSEF 3769 (Table 3). The isolates ARSEF 3769, TPB1, TPB3, and TPB4 were more pathogenic than GHA based on SC_{50} values. However, McGuire et al. (2005) demonstrated significant differences in activity at the two higher doses of conidia of one *L. hesperus* isolate (S44) using analysis of variance, suggesting that at higher rates, the isolate may be more active.

Relative pathogenicity based on mortality among isolates to *L. lineolaris* was more difficult to discern on the basis of LT_{50} values than LC_{50} values. The only LT_{50} value that was significantly lower than GHA was ARSEF 3769 at 1.7×10^4 conidia cm^{-2} (Table 4). Estimates of LT_{50} values were approaching the 10 days incubation time for bioassays with *L. lineolaris* at the two lowest conidia concentrations for mortality and therefore are only presented at the two highest conidia concentrations. Liu et al. (2002) found only three of 32 isolates from six genera to be significantly more pathogenic to *L. lineolaris* than GHA. Two of the three were isolated from Hemiptera and one was isolated from a *Lygus* spp. Although three of the isolates were significantly more pathogenic, the LC_{50} value for the most pathogenic isolate was only 2.8 times lower than the LC_{50} value for GHA. Whereas, half of the LC_{50} values for isolates from *Lygus* spp. in the present study were at least 10 times lower than LC_{50} values for GHA in bioassays against *L. lineolaris*.

At the highest conidia concentration (8.1×10^4 conidia cm^{-2}), LT_{50} values for mortality of *L. hesperus* treated with all isolates from *Lygus* spp. other than WTPB1 and WTPB2 were significantly lower than those treated with GHA. At 8.1×10^3 conidia cm^{-2}, LT_{50} values for all isolates from *Lygus* spp. other than TPB2, WTPB1, and WTPB3 were significantly lower than values for GHA. At the two lowest conidia concentrations for mortality LT_{50} values exceeded the 7 days incubation time of *L. hesperus* bioassays and therefore are only presented at the two highest conidia concentrations.

3.3. Radial growth rates

The isolates selected for this study did not grow at either 35 or 37 °C. No significant differences were observed in growth rates at 28 °C among isolates. However, at 32 °C significant differences in growth rates were observed (Table 5). All of the *L. hesperus* isolates and three of the *L. lineolaris* isolates (ARSEF 3769, TPB2, and TPB3) grew faster than GHA at 32 °C suggesting some adaptation to climate by native isolates. Noma and Strickler (1999) demonstrated better control of *L. hesperus* in alfalfa with Mycotrol (GHA) in pre-bloom (June) trials than bloom (July) trials. They suggested that the lower control in July may have resulted from poor coverage or reduced pathogenicity at higher temperatures. They later demonstrated that *L. hesperus*

Table 5
Growth rate (mm/day) (average ± SE) of *B. bassiana* isolated from *L. hesperus* and *L. lineolaris* at 28 and 32 °C

Isolate	28 °C	32 °C
GHA	1.55 ± 0.03	0.18 ± 0.004 f[B]
3769	1.09 ± 1.06	0.64 ± 0.003 c
TPB1	1.30 ± 0.40	0.32 ± 0.06 ef
TPB2	0.68 ± 0.04	0.44 ± 0.004 de
TPB3	1.69 ± 0.14	0.52 ± 0.007 cd
TPB4	1.52 ± 0.60	0.32 ± 0.009 ef
WTPB1	1.71 ± 0.75	0.46 ± 0.002 d
WTPB2	1.93 ± 0.0006	0.86 ± 0.003 b
WTPB3	1.08 ± 0.61	1.08 ± 0.03 a
F (*df*)	1.96 (8, 17)	34.13 (8, 17)
CVC (SE)[A]		0.14 (0.067)

[A] Critical value for comparison ($P < 0.05$).
[B] Averages followed by a similar letter are not significantly different (protected least significant difference).

Table 4
Mortality (LT_{50} values) for adult *L. lineolaris* and *L. hesperus* after exposure to *B. bassiana* strain GHA, ARSEF 3769, and isolates from *L. lineolaris* (TPB) and *L. hesperus* (WTPB)

Isolate	LT_{50} values[A] (±SE) at two concentrations (conidia cm^{-2})			
	Lygus lineolaris mortality[B]		*Lygus hesperus* mortality[B]	
	1.7×10^4	1.7×10^5	8.1×10^3	8.1×10^4
GHA	7.6 ± 1.0 ab	6.0 ± 0.8 ab	15.7 ± 2.6 ab	6.6 ± 0.7 a
3769	5.2 ± 0.4 c	6.2 ± 0.6 ab	5.3 ± 0.4 e	4.3 ± 0.1 d
TPB1	7.3 ± 0.7 ab	6.0 ± 0.4 ab	5.6 ± 0.4 e	4.6 ± 0.3 bcd
TPB2	7.1 ± 0.6 a	6.2 ± 0.3 ab	10.5 ± 1.4 bcd	4.7 ± 0.4 bcd
TPB3	5.8 ± 0.4 b	6.1 ± 0.5 ab	7.5 ± 0.8 d	4.4 ± 0.4 bcd
TPB4	6.0 ± 0.5 abc	5.6 ± 0.3 b	8.3 ± 0.9 cd	5.0 ± 0.1 b
WTPB1	6.9 ± 0.6 ab	8.8 ± 1.4 a	10.7 ± 1.3 bc	5.2 ± 0.5 abc
WTPB2	6.5 ± 0.6 abc	5.7 ± 0.8 ab	9.5 ± 1.1 cd	5.3 ± 0.4 ab
WTPB3	6.3 ± 0.6 abc	7.9 ± 1.0 a	26.8 ± 7.3 a	4.4 ± 0.1 cd

[A] LT_{50} values for mortality were estimated by survivorship analysis (Proc Lifereg, SAS) using a Weibull distribution and right censored data for >10 days for *L. lineolaris* and >7 days for *L. hesperus*, corresponding to last day of incubation in bioassays. LT_{50} values >10 days for *L. lineolaris* and >7 days for *L. hesperus* are extrapolated beyond the incubation time of the bioassay.
[B] LT_{50} values for *L. lineolaris* control mortality = 14.4 ± 1.0 and *L. hesperus* control mortality = 77 ± 30.

survived treatment with GHA at 35 °C at concentrations that caused 90% mortality at 25 °C (Noma and Strickler, 2000). Isolates in the present study that grew faster at 32 °C may extend the range of *B. bassiana* control into warmer climates. Liu et al. (2002) also compared temperature growth optima for isolates previously found to be more pathogenic to *L. lineolaris* than GHA. Of the five isolates assayed, one isolate (*M. anisopliae* 3540) germinated quickly at 35 °C, whereas all others did not germinate. Although this isolate was found to be only slightly more pathogenic than GHA to *L. lineolaris* (Liu et al., 2002), its ability to germinate at higher temperatures may warrant further investigation for *Lygus* control in cotton IPM.

3.4. Exposure to solar radiation

All isolates were highly susceptible to simulated solar radiation exhibiting LC_{50} values ranging from 13 to 24 J cm^{-2} (Table 6). Isolates from *L. hesperus* demonstrated the greatest survival under simulated solar radiation with LC_{50} values ranging from 19 to 24 J cm^{-2}. Estimates of LC_{50} values among isolates from *L. lineolaris* and GHA were all within 2 J cm^{-2}, ranging from 13 to 15 J cm^{-2}. Explanations for the higher solar stability of *L. hesperus* isolates are unclear; no pigmentation is evident in any of the isolates. However, conditions in the SJV are typically sunny all summer, with little cloudiness and there could be some adaptation to surviving long periods of sunlight. Conditions in the Delta of Mississippi are not as sunny and adaptations may not be as necessary to survival. Selecting isolates with natural resistance to solar radiation may significantly improve mycoinsecticide efficacy under environments of high solar radiation. Additional work is being conducted on formulations for protecting *B. bassiana* conidia from solar radiation and evaluating the contribution of treated plant surfaces to *Lygus* mortality (Leland and Behle, 2004; in press).

Table 6
Effect of artificial sunlight on survival of *B. bassiana* conidia

Isolate	Loss in conidia germination (48 h) vs. exposure (J cm^{-2})				
	Slope ± SE[a]	LC_{50} [b] (J cm^{-2})	95% CL	χ^{2c}	$P < \chi^2$
GHA	1.7 ± 0.3	15	13–18	45	<0.001
3769	1.8 ± 0.3	15	12–20	27	<0.001
TPB1	2.1 ± 0.4	13	10–16	28	<0.001
TPB2	2.6 ± 0.5	13	11–16	23	<0.001
TPB3	2.1 ± 0.4	13	10–16	30	<0.001
TPB4	2.2 ± 0.5	13	10–16	23	<0.001
WTPB1	1.9 ± 0.2	19	16–22	62	<0.001
WTPB2	1.6 ± 0.1	24	21–27	117	<0.001
WTPB3	2.0 ± 0.2	22	20–24	155	<0.001

[a] Slope represents regression of probits of proportion of conidia not germinating versus exposure (J cm^{-2}).
[b] LC_{50} values and 95% confidence limits (CL) estimated by probit analysis (Proc Probit, SAS).
[c] $\chi^2$and $P > \chi^2$values represent the probability of slope $\neq 0$, rather than fit to the Probit model.

3.5. Beauvericin production

Isolates of *B. bassiana* from *L. lineolaris*, *L. hesperus*, and GHA produced a range of beauvericin concentrations in vitro and in vivo (Table 7). Beauvericin was not detected in filtrate of PD-broth from cultures of TPB2, TPB3, TPB4, and WTPB3. Beauvericin concentrations in PD-broth cultures from other isolates ranged from 0.04 (ARSEF 3769) to 0.34 (GHA) pg μL^{-1}. Beauvericin was not detected in all conidia suspensions at 1×10^8 conidia mL^{-1} except TPB3 which had less than 0.02 pgμL^{-1}. Beauvericin concentration in GHA was 0.4 pg μg^{-1}. Beauvericin concentrations in infected *L. hesperus* cadavers was much higher than that observed in vitro, ranging from 22 (ARSEF 3769) to 246 (WTPB1) μg g^{-1}. Two limitations to the analytical methods used for determining beauvericin concentration should be considered when interpreting these data. First, an underlying assumption is that there is quantitative extraction of the beauvericin into the water, or water/acetonitrile and that beauvericin is not left in residual conidia that are lost in filtering or on the column and not measured. This has not been fully studied. Second, while the results are linear and quantitative across the range of interest, the CV for quantification of ion trap MS and MS/MS is fairly high (ca. 25%); so while the data can be quite useful for comparative purposes, it is not highly precise. Nonetheless, precision of detection was sufficiently high to draw conclusions about differences in beauvericin production among isolates. This initial survey of the isolates indicates that beauvericin production in vitro by isolates from *Lygus* spp. is similar to beauvericin production by the EPA- registered GHA.

These data represent an initial examination of beauvericin production that will require additional replication and re-evaluation as production conditions change to ensure that beauverin production does not increase above threshold limits set by the EPA.

Table 7
Beauvericin production by *B. bassiana* strain in vitro and in vivo

Isolate	PD-broth (pg/μL)	Conidia suspensions (pg/μL)	In vivo (μg/g)
GHA	0.34	ND	96.8
3769	0.04	ND	22
TPB1	0.28	ND	132
TPB2	ND[a]	ND	162
TPB3	ND	<0.02	28
TPB4	ND	ND	97
WTPB1	0.18	ND	246
WTPB2	0.14	ND	166
WTPB3	ND	ND	237

[a] ND, beauvericin was not detected in these samples.

3.6. Genetic relatedness

Although not the only tool for determining absolute genetic relatedness, the seven microsatellite (SSR) markers can be used to get a rough estimate of the genetic relatedness of groups of *B. bassiana* isolates. For example, recent papers have used multiple techniques including SSRs (Rehner and Buckley, 2003), PCR-based restriction fragment length polymorphism, SSRs and 28 S rDNA haplotyping, (Wang et al., 2003) and group I intron characterization (Coates et al., 2002) to group isolates of *B. bassiana* (Fig. 1). Clearly, however, isolates from *L. lineolaris* in Mississippi and Arkansas were distinctly separated from isolates from *L. hesperus* in California. One of the isolates from *L. lineolaris* in Mississippi was more closely related to ARSEF 3769 (from *L. lineolaris* in Arkansas) than the other isolates from Mississippi. Isolates from the two *Lygus* spp. were more closely related to each other than to GHA. Isolates TPB1 and TPB2 were identical based on the seven SSR markers used and they were for the most part similar with regards to characteristics measured with the exception of LT_{50} values in bioassays against *L. hesperus*. Collections of these isolates were separated by ≈2 months and in geographic areas separated by ≈16 km. In addition to suggesting relatedness, the markers will also be useful in field tests to determine impact of various isolates on mortality and infection of insects.

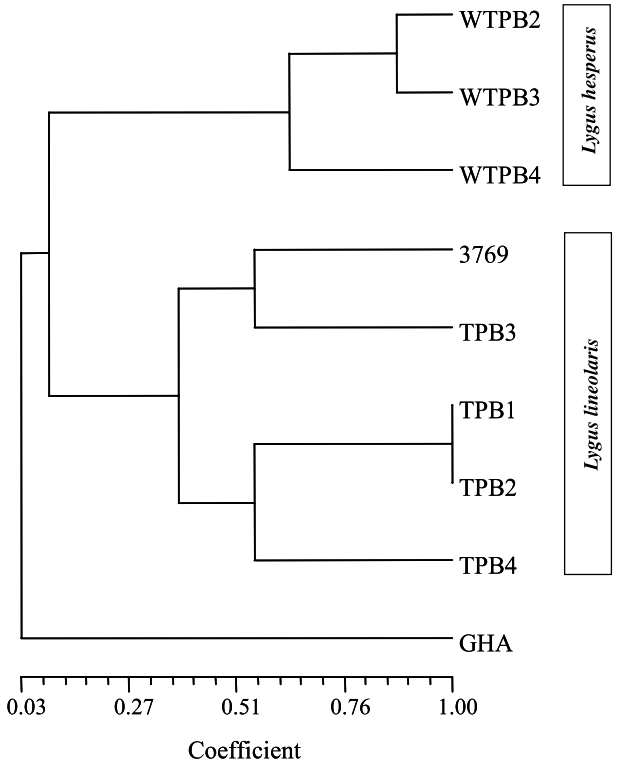

Fig. 1. Dendrogram, based on microsatellite markers, of genetic relatedness of *B. bassiana* isolates from *L. hesperus* from the San Joaquin Valley, California, *L. lineolaris* from the delta region of Mississippi, *L. lineolaris* from Arkansas, and GHA. See Table 1 for isolate information and text for molecular biology procedures.

4. Conclusions

There is a large pool of *B. bassiana* isolates available from wild *L. hesperus* and *L. lineolaris* populations with characteristics of a good mycoinsecticide. Four of the five isolates from *L. lineolaris* were significantly more pathogenic to *L. lineolaris* than GHA based on LC_{50} values, and one of these isolates was significantly more pathogenic than GHA to *L. hesperus*. Initial screening of *B. bassiana* isolates from *L. lineolaris* demonstrated that 11 of the 21 isolates were significantly more pathogenic to *L. lineolaris* than GHA based on LC_{50} values (Leland, unpublished data). Select isolates that are more pathogenic than the commercial isolate (GHA) and can grow faster at 32 °C may be better suited to control *Lygus* in the high temperatures. Many isolates were prolific sporulators on barley agar but it remains to be seen if optimization of biphasic culture for specific isolates can improve conidia yield to levels at least comparable to GHA. Isolates from *L. hesperus* demonstrated some natural resistance to solar radiation relative to the GHA isolate and isolates from *L. lineolaris*. It remains to be seen if the greater tolerance to solar radiation observed in these isolates translates into improved field persistence. This study indicates that isolates from *Lygus* spp. may be a valuable resource for improving mycoinsecticide efficacy for *Lygus* control and serves as a basis for selecting one to two isolates for further study under field conditions. Although pathogenicity was higher in some cases and growth rates were faster at 32 °C, the true test of the new isolates will be in the field. Perhaps slight improvements in activity, growth rates and UV stability will add up to improved field activity.

Acknowledgments

Gordon Snodgrass was consulted for advice related to *L. lineolaris* biology and control. Technical assistance was provided by Danny Ballard, Neal Hudson, Candice Harris, and Maria Garcia in California and Gerald Gibson, Leon Hicks, Tabatha Ramsey, and Lisa Self in Mississippi. Statistical consulting was provided by Deborah Boykin (Midsouth Area Statistician, USDA-ARS, Stoneville, Mississippi). Technical grade product of *B. bassiana* (GHA) was provided by Emerald BioAgriculture (Butte, Montana). We thank Richard Humber for supplying isolates of *B. bassiana* from the ARSEF collection and Luella Castrillo, Cornell University, for advice on single conidia isolation techniques.

References

Bradley, C.A., Black, W.E., Kearns, R., Wood, P., 1992. Role of production technology in mycoinsecticide development. In: Leatham, G.E. (Ed.), Frontiers in Industrial Microbiology. Chapman & Hall, New York, pp. 160–173.

Bradley, C.A., Wood, P.P., Black, W.E., Kearns, R.D., Britton, J., 2002. Solid culture substrate including barley. US Patent Application Number 20020006650.

Coates, B.S., Hellmich, R.L., Lewis, L.C., 2002. Nuclear small subunit rRNA group I intron variation among *Beauveria* spp. provide tools provide strain identification and evidence of horizontal transmission. Curr. Genet. 41, 414–424.

Doberski, J.W., Tribe, H.T., 1980. Isolation of entomogenous fungi from elm bark and soil with reference to the ecology of *Beauveria bassiana* and *Metarhizium anisopliae*. Trans. Br. Mycol. Soc. 74, 95–100.

Finney, D.J., 1971. Probit Analysis, third ed. Cambridge University Press, Cambridge. 3 33 pp.

Goettel, M.S., Inglis, D.G., 1997. Fungi: hyphomycetes. In: Lacey, L. (Ed.), Manual of Techniques in Insect Pathology. Acedamic Press, London, pp. 213–249.

Goodell, P.B., Wright, S.D., Carter, M.W.F., 2000. Managing Western tarnished plant bug (*Lygus hesperus)* in a regional context. In: Dugger, P., Richter, D.A. (Eds.), Proceedings of the Beltwide Cotton Conferences, vol. 2. National Cotton Council, Memphis, TN, pp. 1123–1125.

Grafton-Cardwell, E.E., Godfrey, L.D., Brindley, W.A., Goodell, P.B., 1997. Status of *Lygus* bug and cotton aphid *resistance* in the San Joaquin Valley. In: Dugger, P., Richter, D.A. (Eds.), Proceedings of the Beltwide Cotton Conferences, vol. 2. National Cotton Council, Memphis, TN, pp. 1072–1074.

Jaccard, P., 1908. Nouvelles rescherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat. 44, 223–270.

Leland, J.E., Behle, R.W., 2004. Formulation of the entompathogenic fungus, *Beauveria bassiana*, with resistance to UV degradation for control of tarnished plant bug, *Lygus lineolaris*. In: Dugger, P., Richter, D.A. (Eds.), Proceedings of the Beltwide Cotton Conferences, vol. 2. National Cotton Council, Memphis, TN, pp. 1800–1809.

Leland, J.E., Behle, R.W., 2005. Coating *Beauveria bassiana* with lignin for protection from solar radiation and effects on pathogenicity to *Lygus lineolaris*. Biocontr. Sci. Technol. 15 (3), 309–320.

Leland, J.E., Snodgrass, G.L. Prevalence of naturally occurring *Beauveria bassiana* in *Lygus lineolaris* populations from wild host plants of Mississippi and pathogenicity of *B. bassiana* (GHA) among populations. J. Agric. Urban Entomol. (in press).

Liu, H., Skinner, M., Parker, B.L., Brownbridge, M., 2002. Pathogenicity of *Beauveria bassiana*, *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes), and other entomopathogenic fungi against *Lygus lineolaris* (Hemiptera: Miridae). J. Econ. Entomol. 95, 675–681.

McGuire, M.R., 2002. Prevalence and distribution of naturally occurring *Beauveria bassiana* in San Joaquin Valley populations of *Lygus hesperus* (Heteroptera: Miridae). J. Agric. Urban Entomol. 19, 237–246.

McGuire, M.R., Ulloa, M., Park, Y.-H., Hudson, N., 2005. Biological and molecular characteristics of *Beauveria bassiana* isolates from California *Lygus hesperus* (Hemiptera: Miridae) populations. Biol. Control, 33:307–314.

Milner, R.J., Huppatz, R.J., Swaris, S.C., 1991. A new method for assessment of germination of *Metarhizium* conidia. J. Invertebr. Pathol. 57, 121–123.

Noma, T., Strickler, K., 1999. Factors affecting *Beauveria bassiana* for control of *Lygus* bug (Hemiptera: Miridae) in alfalfa seed fields. J. Agric. Urban Entomol. 16, 215–233.

Noma, T., Strickler, K., 2000. Effects of *Beauveria bassiana* on *Lygus hesperus* (Hemiptera: Miridae) feeding and oviposition. Environ. Entomol. 29, 394–402.

Rehner, S.A., Buckley, E.P., 2003. Isolation and characterization of microsatellite loci from the entomopathogenic fungus *Beauveria bassiana* (Ascomuta: Hypocreales). Mol. Ecol. Notes 3, 409–411.

Rohlf, F.J., 2002. NTSYSpc: Numerical Taxonomy System, version 2.1. Exeter Publishing, Ltd., Setauket, New York, USA.

SAS, 1999. SAS Windows Version 8.1999. Copyright 1999-2001 by SAS Institute Inc., Cary, NC USA.

Snodgrass, G.L., 1996. Pyrethroid resistance in field populations of the tarnished plant bug (Heteroptera: Miridae) in cotton in the Mississippi Delta. J. Econ. Entomol. 89, 783–790.

Snodgrass, G.L., Elzen, G.W., 1994. Efficacy of Naturalis-L® for adults and nymphs of the tarnished plant bug in cotton. In: Dugger, P., Richter, D.A. (Eds.), Proceedings of the Beltwide Cotton Conferences, vol. 2. National Cotton Council, Memphis, TN, pp. 1103–1104.

Snodgrass, G.L., Scott, W.P., 2002. Tolerance to acephate in tarnished plant bug (Heteroptera: Miridae) populations in the Mississippi river delta. Southwest. Entomol. 27, 191–199.

Snodgrass, G.L., Scott, W.P., Robbins, J.T., Hardee, D.D., 2000. Area wide management of the tarnished plant bug by reduction of early-season wild host plant density. Southwest. Entomol. Suppl. 23, 59–67.

Steinkraus, D.C., Tugwell, N.P., 1997. *Beauveria bassiana* (Deuteromycotina: Moniliales) effects on *Lygus lineolaris* (Hemiptera: Miridae). J. Entomol. Sci. 32, 79–90.

Wang, C., Shah, F.A., Patel, N., Li, Z., Butt, T., 2003. Molecular investigation on strain genetic relatedness and population structure of *Beauveria bassiana*. Environ. Microbiol. 5, 908–915.

Williams, M.R., 2004. Cotton Insect Loss Estimates – 2003. In: Dugger, P., Richter, D.A. (Eds.), Proceedings of the Beltwide Cotton Conferences, vol. 2. National Cotton Council, Memphis, TN, pp. 1258–1312.